PB85-212132

Some Methods for Measuring Effects of Toxicants on Laboratory- and Field-Colonized Estuarine Benthic Communities

(U.S.) Environmental Research Lab.
Gulf Breeze, FL

Jun 85

U.S. Department of Commerce
National Technical Information Service
NTIS

PB85-212132

EPA/600/D-85/120
June 1985

SOME METHODS FOR MEASURING EFFECTS OF TOXICANTS ON LABORATORY- AND FIELD-COLONIZED ESTUARINE BENTHIC COMMUNITIES

by

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TECHNICAL REPORT DATA
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1. REPORT NO. EPA/600/D-85/120	2.
3. RECIPIENT'S ACCESSION NO. PB85 212132/AS	
4. TITLE AND SUBTITLE SOME METHODS FOR MEASURING EFFECTS OF TOXICANTS ON LABORATORY- AND FIELD-COLONIZED ESTUARINE BENTHIC COMMUNITIES	5. REPORT DATE June 1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) M.E. Tagatz	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS same as #12	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory - Gulf Breeze, FL Office of Research and Development U.S. Environmental Protection Agency Gulf Breeze, FL 32561	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA/600/4
15. SUPPLEMENTARY NOTES	

16. ABSTRACT

Effects of toxicants on estuarine macrobenthic animals that developed in sand-filled boxes in the laboratory and field during 8 weeks were determined by comparing community structures in control boxes and in boxes treated with a toxicant. Communities that developed were diverse and averaged 1441 individuals, 30 species, and 6 phyla for laboratory tests and 933 individuals, 51 species, and 8 phyla for field tests. Toxicants were introduced via water, air, or sediment and before, during, or after colonization. Tests with laboratory- and field-colonized communities provided corroborating data as well as data unique to each test. Various structural attributes among laboratory, experimental field, and natural field communities were similar, indicating that data derived from the laboratory and field toxicity tests can have good environmental applicability.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 24
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE $7.00

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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ABSTRACT: Effects of toxicants on estuarine macrobenthic animals that developed in sand-filled boxes in the laboratory and field during eight weeks were determined by comparing community structures in control boxes and in boxes treated with a toxicant. Boxes were colonized in the laboratory by planktonic larvae in continuously supplied unfiltered seawater and in the field by animals that occurred naturally. Field boxes were placed in estuarine waters, either near the laboratory or at salt-marsh sites subjected to contamination by mosquito control pesticide applications. Eight separate studies were conducted using the same test materials in laboratory and field tests. Communities that developed were diverse and averaged 1441 individuals, 30 species, and 6 phyla for laboratory tests and 933 individuals, 51 species, and 8 phyla for field tests. Toxicants were introduced via water, air, or sediment and before, during, or after colonization. Tests with laboratory- and field-colonized communities provided corroborating data as well as data unique to each test. Various structural attributes among laboratory, experimental field, and natural field communities were similar, indicating that data derived from the laboratory and field toxicity tests can have good environmental applicability.

KEY WORDS: tests, environmental tests, field tests, test equipment, toxicity, water pollution, benthos, aquatic animals, aquatic toxicology, community structure

Scientists at the U.S. Environmental Protection Agency Environmental Research Laboratory, Gulf Breeze, Florida, use benthic community tests to aid in assessment of toxicant impact on the estuarine environment. Results from community tests can have environmental applicability since they provide for interspecific interactions and allow more species of representative phyla to be exposed than do single-species toxicity tests. A laboratory test that assessed the impacts of six substances on developing communities of benthic estuarine organisms was described by Hansen and Tagatz [1]. Eight separate tests to determine effects of substances on field-developed benthic communities for comparison with eight tests on laboratory-developed communities using the same substances also have been completed [2-9]. Substances tested included pesticides, industrial chemicals, and drilling muds. This paper summarizes the methods and results of those field tests and describes recent modifications of the earlier laboratory test. Objectives of dual testing, i.e., laboratory and field, were to obtain a larger and more diverse data base to evaluate toxicity, to compare types and sensitivities of animals in laboratory and field communities, and to use the field test to better simulate natural conditions and to indicate applicability of the laboratory results to the field.

METHODS

The timing of tests and the manner in which the test material was applied varied. Some laboratory and field tests were conducted concurrently while others took place in different seasons. In this paper, toxicant exposures in field studies are divided into experimentally controlled (direct exposures) and those that resulted from spray applications administered for mosquito control (environmental exposures). Unless otherwise indicated, nominal concentrations of each test material are reported for all tests.

Laboratory-Colonized Communities

Our laboratory system allowed planktonic larvae in flowing seawater to colonize sand-filled contaminated and uncontaminated test containers for approximately eight weeks. In the original design used for each treatment [1], a rectangular constant-head box straddled ten adjacent narrow test containers. Seawater entered one end of the head box, flowed across to supply the containers via ten standpipes, and the excess left the opposite end of the head box. To eliminate any "end of the row" differences among test containers and any position difference in the number and type of larvae flowing along the length of the constant-head box, this design was modified to four [10], six [11], or eight [12] containers extending radially from a centrally located inflow of seawater. Although all the radial designs were used sucessfully in conjunction with field tests, the four-container design -- containers here referred to as boxes -- is emphasized because it incorporates the same type of box as was used for field-colonized communities (Fig. 1). Two four-box designs are described for each treatment since eight, rather than four replicates, separate toxicant effects more readily from other community influences, such as particularly heavy and localized reproduction or predation.

Boxes used for a control and for each of three concentrations of a toxicant were 32 cm x 32 cm x 6 cm deep. They were constructed of acrylic plastic and filled with clean silica sand (particle size 0.2 to 0.8 mm) dredged four or more months previously from Santa Rosa Sound, Florida. Each treatment consisted of four boxes placed in each of two frame containers that, when filled, contained a 3-cm layer of water over the sand (Fig.1). Unfiltered seawater from Santa Rosa Sound flowed to an overhead splitter box from which each of eight adjacent standpipes supplied

water through a larger glass tube to the center of each frame container. Flow rate was maintained at 1.5 litres/min by adjusting the height of each standpipe. Water flowed from the frame container through notched openings on all sides. Illumination consisted of alternating 12-h periods of light and darkness.

Depending on the test, toxicants were added to the sand prior to colonization, or to the water during colonization or after assemblages of animals had developed. Water exposures during colonization were intermittent or continuous. Three concentrations of each toxicant were tested and usually differed by an order of magnitude. When introduced to sand, toxicant was hand-mixed with the upper portion or entire depth of sand in the test container or added as a layer over the surface of the sand. When introduced to the water column, each toxicant was metered by pump through flexible tubing and injected through a side opening in the glass tubing that carried seawater to each frame container. Intermittent exposures at two- or four-week intervals were used to simulate concentrations and frequency of fenthion applications used in the field for mosquito control [8]. In some tests, introduction of the toxicant was concurrent with the beginning of colonization to determine its effect on settling of benthic organisms [4]. We felt that continuous exposure of early larval forms would provide a more sensitive measure of toxicity than exposure of later developmental stages. In other tests, toxicant was introduced at eight weeks, after assemblages of animals had developed so that exposure regime would be equivalent to that used for some field-colonized communities [5]. Duration of the later exposures ranged from 6 to 14 days. By exposing an established community to a toxicant, mortality could often be empirically determined to document toxicant effect; in

exposure of early larval forms, mortality was inferred by the absence of individuals.

Communities were harvested at the end of the test by washing sediments through a 1-mm mesh sieve. Macroinvertebrates retained were relaxed (menthol and magnesium sulphate in water), preserved in formalin or alcohol, identified, and counted. One-mm mesh was preferred over a smaller size because fewer and larger animals that were retained and the absence of sand expedited collection and identification of the animals. However, mesh size for sampling and analyzing marine macrobenthos should not exceed 1.0 mm [13].

Field-Colonized Communities

Direct Exposures -- The effects of direct toxicant exposure on macrobenthic animal communities that colonized sand-filled boxes in the field were determined by exposing them via the sand, or by bringing the established communities into the laboratory for exposure via the water. Field boxes were the same size and filled with the same type of sand used in laboratory tests. Four to eight replicates were used for a control and for each concentration of a toxicant. Boxes were numbered for identification and were placed by SCUBA divers in 3 m of water in Santa Rosa Sound, adjacent to our laboratory. They were lowered and raised, in groups of four, by use of a metal holding frame connected by cable to a winch aboard the dive boat. Divers placed individual covers on the boxes during transfer between laboratory and field and positioned them in the substratum so that their surfaces were level with the surrounding sand. This not only presented an environmentaly realistic appearance but also resulted in little or no removal of sand from the containers as the result of scouring action of waves or currents. Initial use of metal

screen (6.4-mm square mesh) covers to exclude predation by large fish and crabs was discontinued when it was determined that these predators did not drastically affect community structure in uncovered boxes. Boxes were colonized for approximately eight weeks by settling larvae or by immigration.

Toxicants were added to the sand prior to colonization [6] or to the water at the end of eight weeks of colonization when communities were brought into the laboratory and placed in frame containers for exposure in the same manner as laboratory-colonized communities [5].

At the end of the test, field communities were harvested in the laboratory. Animals retained by a 1-mm mesh sieve were relaxed, preserved, identified, and counted by using the same procedures as for laboratory communities.

Environmental Exposures -- The effects of fenthion on macrobenthic animals colonizing sand-filled boxes in two salt-marsh tidal lagoons adjacent to Santa Rosa Sound were determined after two mosquito control sprayings in a five-day period [8]. The lagoons were within an area that received ground applications of fenthion for control of adult mosquitoes during the mosquito season. Eighteen sand-filled boxes were placed in each lagoon in approximately 0.5 m of water and positioned so that their surfaces were level with the surrounding sand.

Benthic animals were allowed to colonize the sand in the boxes for eight weeks before application of fenthion. One-half of the boxes at each site served as a control and were harvested two days before the first fenthion spray; the remaining boxes were harvested two days after the second spray. The control boxes were placed in the field one week earlier than those exposed to fenthion so that colonization time would be similar.

Communities were harvested by emptying the contents of each box into

a pail with a nylon-screen bottom (1-mm mesh), agitating the screen in the lagoon water, and removing the screen with retained material from the bottom of the pail. The screen was formed into a bag to hold the retained material for processing through an animal relaxer (propylene phenoxetol in water) and for fixing in formalin that contained Rose Bengal stain. Preserved animals were identified and counted in the laboratory.

CRITERIA FOR EFFECTS

A great variety of measurements can be used to indicate the effects of substances on community structure and function or on community habitat. In our studies, however, impact on macrobenthic communities was determined primarily by comparing numbers of individuals and numbers of species of animals in contaminated and uncontaminated boxes. Sufficient numbers of animals were collected in each of the eight studies (Table 1) for analysis of variance and Duncan's post hoc multiple comparison procedure [14]. Results were presented as pooled data, by phylum, from the control and for each concentration of the toxicant [2-9]. Various biological indices for species density, diversity, and dominance were also applied to our laboratory- and field-derived data to characterize differences in community structure. These included the Shannon-Weaver index of diversity, Simpson's index of dominance, Bray-Curtis dissimilarity index, rarefaction diversity index, and Spearman's measure of rank correlation [6,10,15]. Numbers of species and individuals usually were more sensitive and reliable indicators of stress than were the biological indices employed since they indicated toxicant effect at lower concentrations [6, 15].

Redox potential profiles of sediments were determined in some tests for comparison among treatments. Contamination by compounds such as creosote

and dibutyl phthalate resulted in a more anaerobic and chemically reducing environment than in the control [6,9].

In contaminated sediment studies, cores were analyzed to determine persistence of the toxicant. For example, it was found that initial concentrations of creosote in sand decreased by 30 percent in the laboratory and by 42 percent in the field after eight weeks [6]. Creosote was not detected in water samples from the laboratory test after about three weeks, also indicating limited leaching from contaminated sediments.

Additions of clay-like substances, such as barite and drilling mud, have shown that these community tests can provide a sensitive measure of the impact of changes in sediment granulometry on benthic animals. Some effects of these substances, mixed with or layered over the surface of the sand, were attributed to physical alteration of the substrate, rather than to direct toxicity [7,16,17].

Some measures of toxicity and of extent of recovery after exposure that were used in community tests conducted only in the laboratory also apply here. Toxicant effect on growth, such as diameter of tunicates or length of pelecypods, was found to be a sensitive parameter [12,15]. In three of the other studies, effects on meiofauna as well as on macrofauna were investigated [1]. Measurements of impact included meiofaunal biomass, density, species composition, and feeding types. In another laboratory study, extent of recovery of macrobenthic communities was determined after animal abundance was lowered by pentachlorophenol [11]. Seven weeks after water-borne exposure was discontinued, total numbers of individuals and species in previously contaminated and control communities no longer differed. However, there were differences from control in relative abundance of species that could be attributed to earlier toxicant exposure.

COMPARISON OF LABORATORY- AND FIELD-COLONIZED COMMUNITIES

Structure

Laboratory-colonized communitites shared many attributes with field-colonized communities [10]. Both consisted primarily of animals from four phyla: Annelida, Mollusca, Arthropoda, and Chordata (Table 2). In addition, a few species represented more than one-half of the total individuals in each community. In some instances, the more abundant species within phyla were the same for the laboratory and field. Usually, about one-fourth of the species were common to both sites. However, laboratory communities averaged more individuals, but fewer species and phyla, than did field communities (Table 1). Because natural assemblages of benthic animals varied seasonally, structural difference between laboratory and field communities was most pronounced when testing occurred at different times. When species density, diversity, and dominance were determined in laboratory and field tests conducted concurrently, they were similar in both environments in two of three studies [6, 10].

In addition to similarities in community structure between laboratory and field boxes, experimental communities were considered representative of animals that concurrently colonized natural areas. Community differences would be expected since settling animals in nature become incorporated into a wide variety of already established natural communities. However, core and dredge samples indicated that macrobenthic organisms near field boxes in Santa Rosa Sound [1, 10] and those at the mosquito-control spray-site [8] consisted of the same dominant phyla and of similar species as in the newly settled experimental boxes.

Toxicity

Both laboratory- and field-derived benthic communities contained

sufficient animals to allow determination of toxicant effects on a variety of macrofauna. In addition to effects based on pooled data by phylum, individual abundances of 28 species from 7 phyla were significantly affected by one or more of the toxicants tested in community tests [1-7, 12, 15-17]. These consisted of 1 tunicate, 1 lancelet, 2 crabs, 1 shrimp, 2 amphipods, 1 barnacle, 5 pelecypods, 3 gastropods, 1 sea cucumber, 1 sea anemone, 9 polychaete worms (6 different families), and 1 nemertean.

Tests using laboratory- and field-colonized communities provided corroborating data on toxicant effects as well as data peculiar to each test. For example, amphipod abundance was significantly affected by fenvalerate in both laboratory and field situations; however, lancelets very sensitive to the chemical in field-colonized communities did not occur in laboratory-colonized communities [3]. Comparisons of phyla most sensitive in laboratory and field tests also show similarities and differences (Table 3). Many differences could be attributed to species specificity within phyla. Concentrations of toxicant that affected macrobenthic abundance usually were the same for laboratory- and field-colonized communities (Table 4). When concentrations differed, animmals colonized in the laboratory were the more sensitive.

Data from our laboratory community tests using fenthion suggested that significant mortality would not occur among macrobenthic animals exposed in the field to multiple sprays of the chemical if resulting water concentrations were $\leq$ 1.4 ppb [8]. Therefore, it was not surprising that there was no significant impact on experimental communities in salt marsh lagoons after two exposures to measured concentrations $\leq$ 0.7 ppb. Our laboratory studies also indicated that a fenthion concentration of 14 ppb could significantly affect the composition of field communities.

However, this concentration did not occur in typical ULV ground sprays of fenthion for control of adult salt marsh mosquitoes and was equivalent to an unrealistically high 80 percent fallout in water, based on the maximum recommended application rate [8].

COMPARISON OF RESULTS OF COMMUNITY AND SINGLE SPECIES EXPOSURES

Where comparative data were available, the phylogenetic groups that were sensitive to the toxicants tested in laboratory community tests were generally similar to those in acute tests with single species. For example, concentrations of fenvalerate and chlorpyrifos were particularly toxic to crustaceans, Corophium acherusicum in community studies and Mysidopsis bahia in acute tests [3,4]. Mollusks were not adversely affected by fenvalerate in either type test [3], but the effect of chlorpyrifos on mollusks in the community test could not have been predicted from acute testing information [4]. Numbers of molluscan larvae settling in our laboratory-colonized communities were affected by 0.1 µg/litre chlorpyrifos; however, the acute effect concentration for oyster larvae was nearly 2000 µg/litre. Hansen and Tagatz [1] reported that the species typically impacted by six substances in the laboratory benthic community test were representative of phyla that were also affected in single-species tests.

In some instances, there were little or no acute test data for comparison with community test data. No information on responses of saltwater animals acutely exposed to dibutyl phthalate was available for comparison with effects on benthic community animals [5]. In addition, comparative acute and community-derived data were scarce for community animals exposed to sediment-bound substances because there is no standard

test for a single species marine benthic invertebrate and sediment.

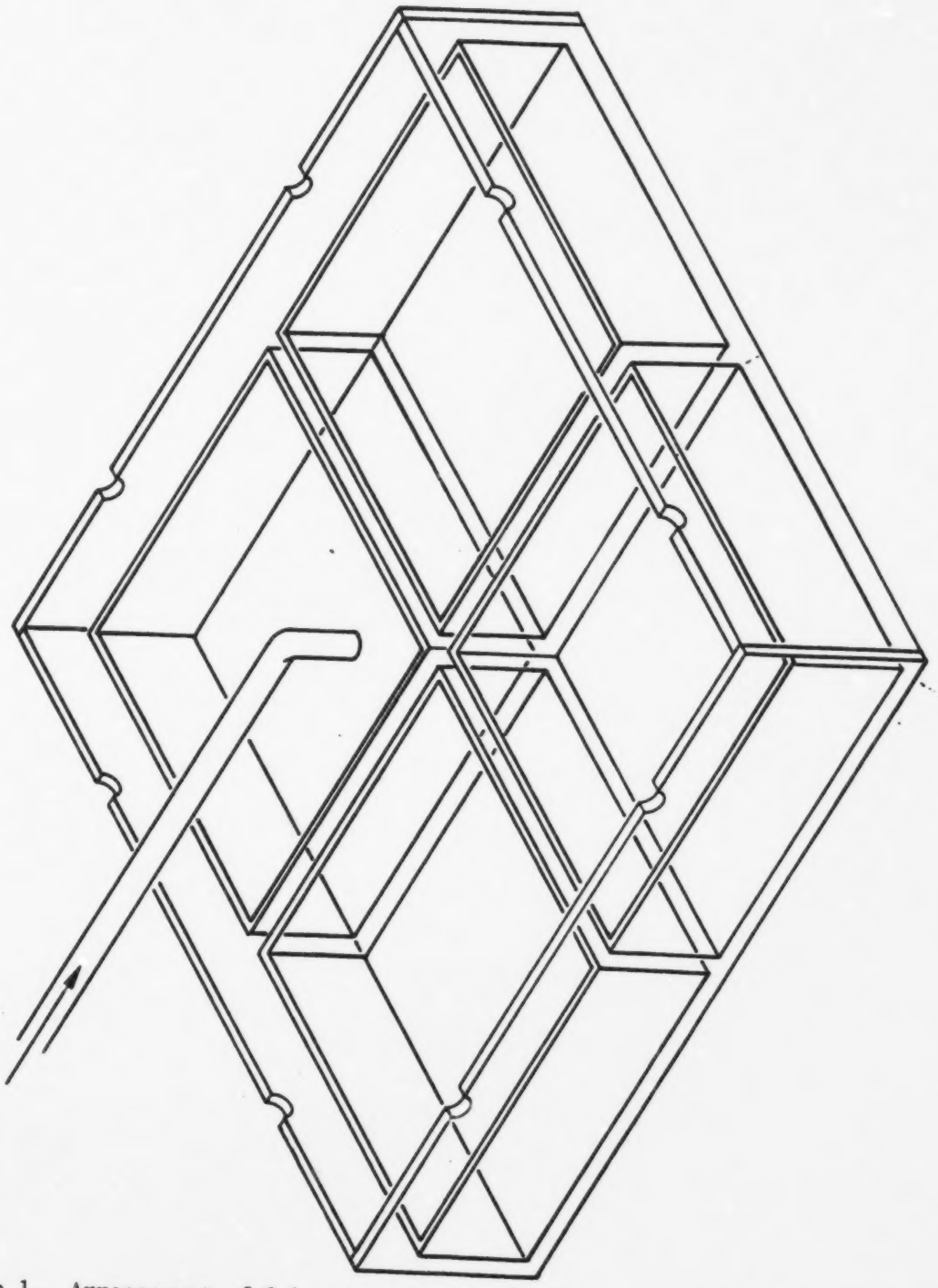

Figure 1. Arrangement of laboratory boxes, four per frame container, to test the effects of toxicants on macrobenthic communities. Seawater was continuously supplied at 1.5 litres/min to the center.

Table 1 - Number of individuals, species, and phyla in laboratory-colonized and in field-colonized benthic communities from eight toxicity studies.

Toxicant	Reference	Laboratory			Field		
		Individuals	Species	Phyla	Individuals	Species	Phyla
Water exposures:							
Chlorpyrifos	4	2003	29	7	2089	65	9
Dibutyl phthalate	5	2331	29	6	181	46	7
Fenthion	8	3129	24	6	642	25	4
Fenvalerate	3	1758	35	6	1462	59	7
Pentachlorophenol	2	800	24	5	346	32	6
Sediment exposures:							
Creosote	6	367	33	5	875	60	10
Drilling mud	7	433	24	6	1081	63	10
Dibutyl phthalate	9	706	40	7	784	58	9
Average		1441	30	6	933	51	8

Table 2 - Average dominance (by rank of individuals) of phyla in laboratory- and field-colonized benthic communities from eight toxicity studies.

Phylum	Rank	
	Laboratory	Field
Mollusca	1	2
Chordata	2	4
Annelida	3	1
Arthropoda	4	3
Coelenterata	5	6
Echinodermata	6	6
Rhynchocoela	7	5
Phoronida	-[a]	7
Hemichordata	-	8
Sipunculida	-	8
Platyhelminthes	-	9

[a] None found.

Table 3 - Most sensitive phyla in laboratory- and field-colonized communities exposed to various toxicants.

Toxicant	Laboratory	Field
Water exposures:		
Fenvalerate	Arthropoda	Arthropoda, Chordata
Pentachlorophenol	Mollusca, Annelida	Mollusca
Chlorpyrifos	Mollusca, Arthropoda	Annelida
Dibutyl phthalate	Arthropoda	Mollusca
Fenthion	None	None
Sediment exposures:		
Creosote	Echinodermata	Annelida, Arthropoda
Drilling mud	Coelenterata, Annelida	Mollusca
Dibutyl phthalate	Echinodermata	Mollusca

Table 4 - Concentrations of toxicant affecting macrobenthic animal abundance in laboratory- and field-colonized communities.

Toxicant (Exposure)	Laboratory	Field
Fenvalerate (water)	0.1 ppb	0.1 ppb
Pentachlorophenol (water)	150 ppb	150 ppb
Creosote (sediment)	177 ppm	177 ppm
Drilling mud (sediment)	10%	10%
Chlorpyrifos (water)	0.1 ppb	1 ppb
Dibutyl phthalate (water)	0.5 ppm	5 ppm
Dibutyl phthalate (sediment)	1000 ppm	1000 ppm
Fenthion (water)	[a]	[a]

[a]No effect at 1.4 ppb (nominal) in the laboratory and at 0.7 ppb (measured) in the field.

CONCLUSIONS

These tests allowed determination of effects of toxicants, or clay-like substances that altered sediment granulometry, on the development of benthic animal communities. The tests are versatile because toxicant can be introduced into the water, air, or sediment, and it can be added before, during, or after colonization. Experimental boxes can also be positioned where they can be contaminated by a toxicant that is applied to the natural environment for a specific purpose. Selection from various experimental procedures (toxicant- and site-specific) may be more realistic for testing the many types of toxicants and environmental threats than development and use of any standard benthic community test.

Tests with laboratory- and field-colonized communities provided corroborating data as well as data peculiar to each test. The laboratory test often was a more sensitive measure of toxicity because more early life stages were exposed; the field test appeared to better represent natural assemblages of animals. Kind and number of animals in experimental communities showed great seasonal variation. However, even when colonized at different times, laboratory and field communities shared many structural attributes and both were representative of animals concurrently colonizing natural areas. Effects observed in laboratory community tests can be predictive of those that occur in field community tests. Results from field tests can be indicative of those that would occur in similar habitats in nature and also delineate limitations on environmental applicability of laboratory data.

Although results from field testing and sampling provided information on how well laboratory-colonized communities simulated natural communities, field validation of laboratory communities was beyond the intended scope

of these studies. Precise determination of the capacity of the laboratory benthic test to simulate natural communities and to predict response to toxicant is dependent on uniformity (time and methods) between laboratory and field testing, coupled with intensive sampling of the natural benthos to indicate spatial and seasonal variability. Sampling the benthos in sea grass and soft bottom environments also could indicate how predictive results from bare sand communities would be to other habitats.

Few comparisons of the effects of toxicants on saltwater animals in acute and community tests could be made on a species basis because the species for which we have acute toxicity data did not commonly occur in experimental benthic communities. More of the dominant species in both laboratory- and field-colonized communities need to be tested acutely to best determine if animal sensitivity to toxicants differs when tested singly or in a community situation.

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